The challenges of ecotox testing of nanomaterials and the BPR

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ABSTRACT: The European Biocidal Product Regulation (BPR) requires dedicated risk assessment of nanomaterials. When it comes to ecotoxicological testing of nanomaterials, meeting these requirements is especially challenging. Overall, these challenges fall into four overall categories: 1) materials characterization, 2) exposure preparation, 3) monitoring stability and 4) monitoring time. In this paper, the challenges are presented and discussed. There is no easy manner in which to deal with the challenges related to ecotoxicological testing of nanomaterials in the light of the BPR requirements. It short the current answer seems to be describe, characterize and document. Characterization is vitally important and has to be done using multiple methods on the nanomaterials as received, in the test media with and without the organisms.

INTRODUCTION

In 2013, the European Biocidal Product Regulation (BPR) went into force (1). The BPR is groundbreaking when it comes to nanomaterials in many ways. It is first when it comes to requiring a separate dossier with all data requirements to be prepared for nanomaterials as well as a dedicated risk assessment when a nanoform of a substance is used.

Under the BPR, producers/importers of biocidal active substances or products need to submit a dossier to the European Chemical Agency containing information on the applicant (i.e. the manufacturer or the importer), the identity and physio-chemical properties of the substance, physical hazards and respective characteristics, method of detection and identification, effectiveness against target organisms, intended uses and exposure, toxicological profile for humans and animals, ecotoxicity studies, environmental fate and behaviour, measures necessary to protect humans, animals and the environment and information on classification, labelling and packaging (2).

Multiple OECD technical guidelines exist on how to do ecotoxicological testing. In 2009, the OECD published a preliminary review of the current OECD test guidelines for their applicability to nanomaterials. In total 24 ecotoxicity testing guidelines were reviewed with the aim to evaluate their adequacy in addressing nanomaterials and in order to identify the need for development of new test guidelines or a revision of the existing test guidelines (3). The OECD found that the basic toxicological principles and the test endpoints were adequate for the testing of nanomaterials, and the recommendation is therefore not an extensive modification of all OECD guidelines, but rather to address the specific issues related to testing of nanomaterials in a separate document. It was also highlighted that the terminology used in the existing guidelines in many cases was not applicable for nanomaterials, and these terms needs to be revised as well (3).

This means that meeting the BPR requirements is a challenging task when it comes to nanomaterials, especially when it comes to ecotoxicological testing of nanomaterials. Overall, these challenges fall into four overall categories: 1) materials characterization, 2) exposure preparation, 3) monitoring stability and 4) time.

MATERIALS CHARACTERIZATION

It is general dogma in traditional toxicology and ecotoxicology of chemicals that “the dose by mass makes the poison”. However, we know that when it comes to the ecotoxicity of nanomaterials it is clear that this is somehow related to the specific properties of the nanomaterials. A range of different properties has been suggested, but few candidates that could explain the ecotoxicological potential of nanomaterials have been systematically tested and fewer have been tested experimentally. In order to identify the property or combination of properties that determine nanomaterial ecotoxicity, we would ideally like to know the size of the primary nanoparticles, size distribution, particle shape, crystal structure, surface chemistry, surface charge, solubility and the state of aggregation and agglomeration (4). Determining all of these properties can be very challenging both from a scientific as well as a technical standpoint and this is especially the case for nanomaterials that are non-spherical and that tend to aggregate and agglomerate in complex media such as artificial algal media and river water. Furthermore, most nanomaterials are non-spherical and tend to aggregate and agglomerate in complex media (5). There is no one method that can answer all of our requirements and it is important that multiple characterization methods are used to characterize the nanomaterials in regard to, for instance, size, shape, zeta potential, state of aggregation and agglomeration and state of dissolution. Methods that can be used to do this characterization include among other electron microscopy, field flow fractionation and dynamic light scattering (4, 5).

EXPOSURE PREPARATION

Exposure preparation represents another significant challenge when it comes to the ecotoxicological testing of nanomaterials. As exposure preparation happens before the test organism is actually exposed to the nanomaterials and the actual ecotoxicological test is performed, it can seem strange that this actually represents a challenge at all. A number of different methods exist when it comes to exposure preparation such as sonication, use of solvents, stirring and encapsulation. It has, however, repetitively been found that the methods used to prepare the nanomaterials and get them into dispersion affects the outcome of the subsequent ecotoxicological testing (6). Although, a lot of research is going on in this regard, the most regulatory and/or environmentally relevant exposure preparation method and approach still has to be determined. Key part of such a method has to be preparing the stock concentration as low as possible in order to minimize aggregation and agglomerate and as close to the highest tested concentration as possible.

Monitoring stability and time

Monitoring stability and time are the final two significant challenges when it comes to ecotoxicological testing of nanomaterials. Nanomaterials seem to undergo a number of changes in regard to stability, aggregation and agglomeration in the duration of the ecotoxicological tests that can last e.g. 48-96 hours. The extent of these changes is known to be effected by time, the composition of the media, the concentration of the nanomaterial used in the test, but clear patterns have still to emerge. Therefore, it is important that a stability study of the nanomaterials in the test media is an integrated part of the test procedures and the chosen dispersion method has be justified and specific settings and procedures reported. For example, the exact information on aperture power output (W) and amplitude (%) of the sonicator should be reported if sonication is used (7).

ADDRESSING ECOTOXICOLOGICAL CHALLENGES OF TESTING NANOMATERIALS

The OECD has published a guidance document on sample preparation and dosimetry in 2010, and this was reviewed and amended in 2012 (8). The guidance, however, is still not an actual test guideline with specific steps and requirements, but rather an outline of important considerations that researchers and potential BPR-registrant should bear in mind in order to obtain meaningful and reproducible test results. The lack of clear and specific guidance on how to complete nano-specific testing creates a significant challenge for registrants regarding how they can justify they have completed nano-specific testing and have completed the specific risk assessment of the nanomaterial in the biocidal product that they wish to register? Similarly, how can the competent authorities assess the appropriateness of the submitted data and whether the risk assessment of the bulk form of a given active substance cover the nanoform as well?

There is no easy manner in which to deal with the challenges related to ecotoxicological testing of nanomaterials in the light of the BPR requirements. It short the current answer seems to be describe, characterize and document. Anyone that engages in ecotoxicological

testing of nanomaterials should start with the basics and describe the nanomaterial in regard to colour, form, etc. including how the materials are received, handled, stored, etc. as this might affect the properties of the nanomaterial and the subsequently observed ecotoxicity. Characterization is vitally important and has to be done using multiple methods on the nanomaterials as received, in the test media with and without the organisms and using more than just one method. As a minimum concentration by mass, dissolved fraction, zeta potential and the size distribution should be reported. In the process of applying multiple characterization methods, it is vital to understand, describe and reflect on their pros and cons and hope they can collectively can bring us closer to understand how the properties of the nanomaterial affect their ecotoxicological potential.

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